SUPPLEMENTAL DATA

LncRNA Tug1 Regulates Mitochondrial Bioenergetics in Diabetic Nephropathy

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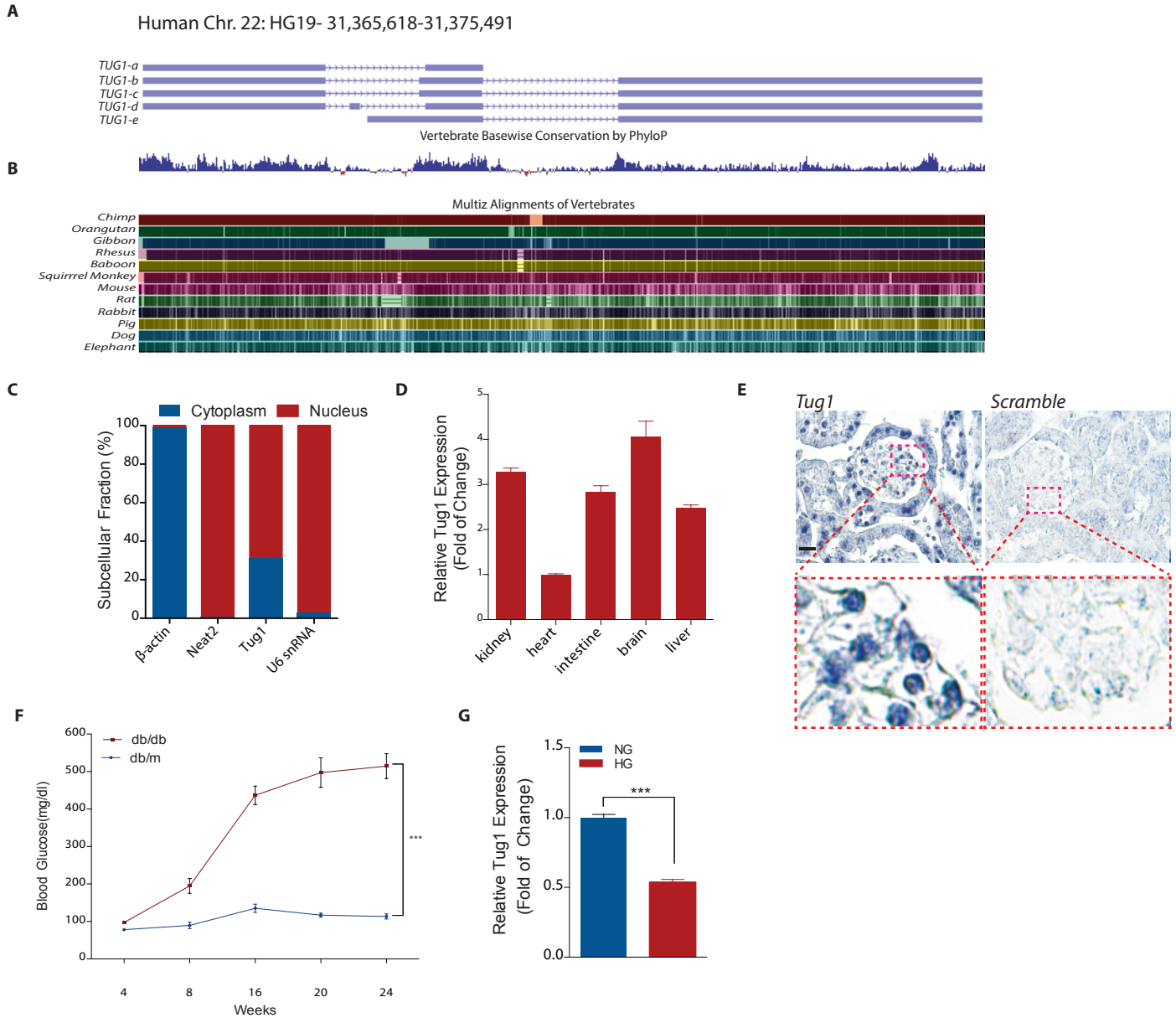
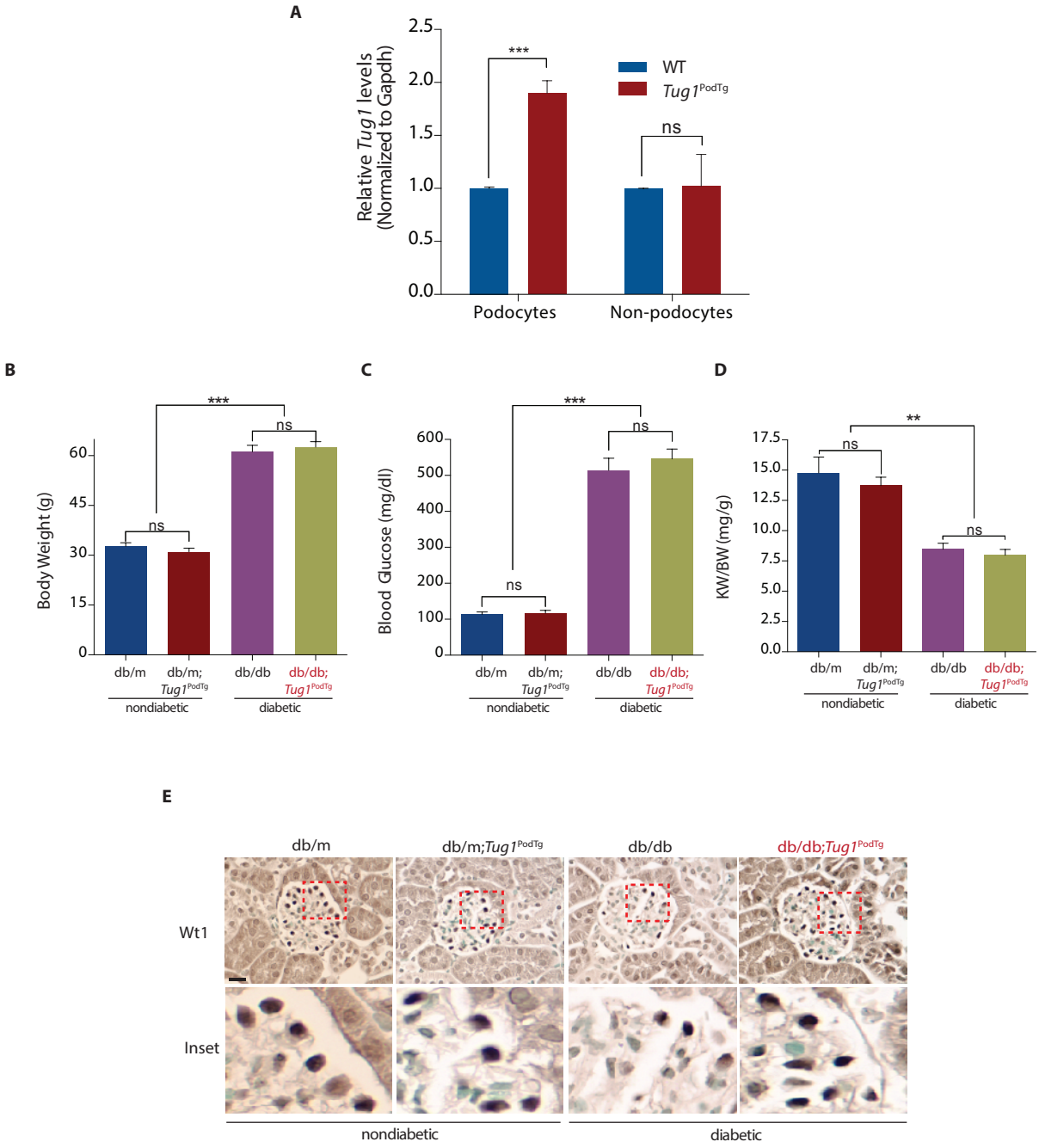
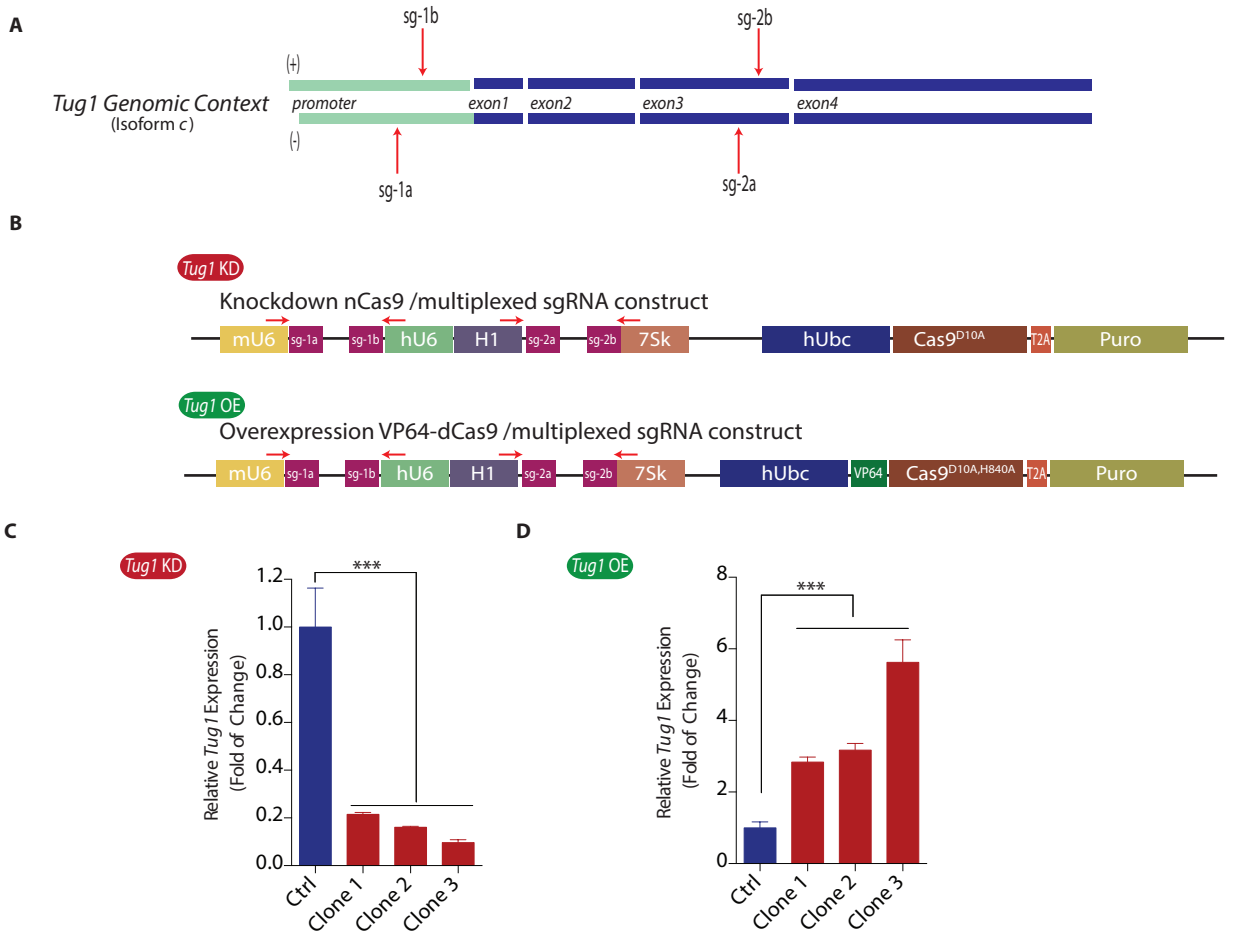


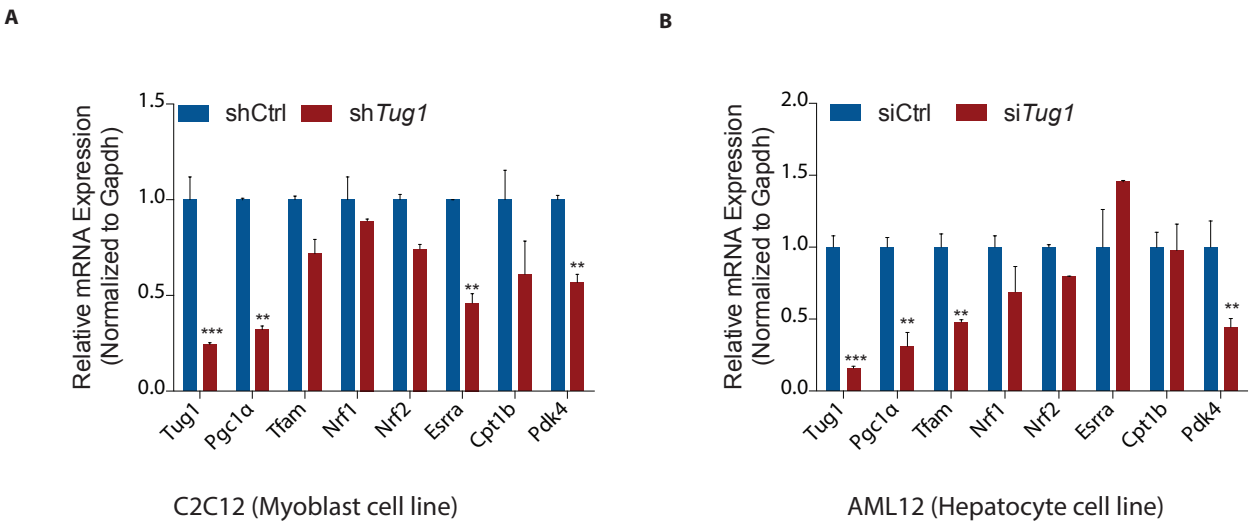
Supplemental Figure 1. *TUG1* Genomic Context, Conservation and Characteristics. A) Exon structure of the five human *TUG1* isoforms located on human chromosome 22, mice contain three isoforms with similar exon strcuture (**B)** Evolutionary conservation between human *TUG1* and several vertebrate species as denoted. (**C)** Cellular fractionation studies to determine percentage localization of *Tug1* RNA. Controls include the cytoplasmic localized β-actin mRNA, Neat2 lncRNA and the U6 small nuclear RNA (snRNA). **(D)** RNA levels of *Tug1* in different mouse tissues. **(E)** Light microscope images of chromogenic RNA in situ hybridization using LNA-modified *Tug1* specific or scramble, non-specific probes in kidney sections from adult mice. Zoom inset highlights glomerular expression and podocyte localization of *Tug1* RNA. Scale bars denote 50μm. (**F)** Fasting blood glucose level of mice in the indicated groups at different time points. **(G)** RNA levels of *Tug1* in mouse podocytes cultured under normal glucose (NG, 5mM) or high glucose (HG, 25mM) conditions for 48 hours. Expression values in D and G were normalized to Gapdh. Data are presented as mean±s.e.m. ***P<0.001



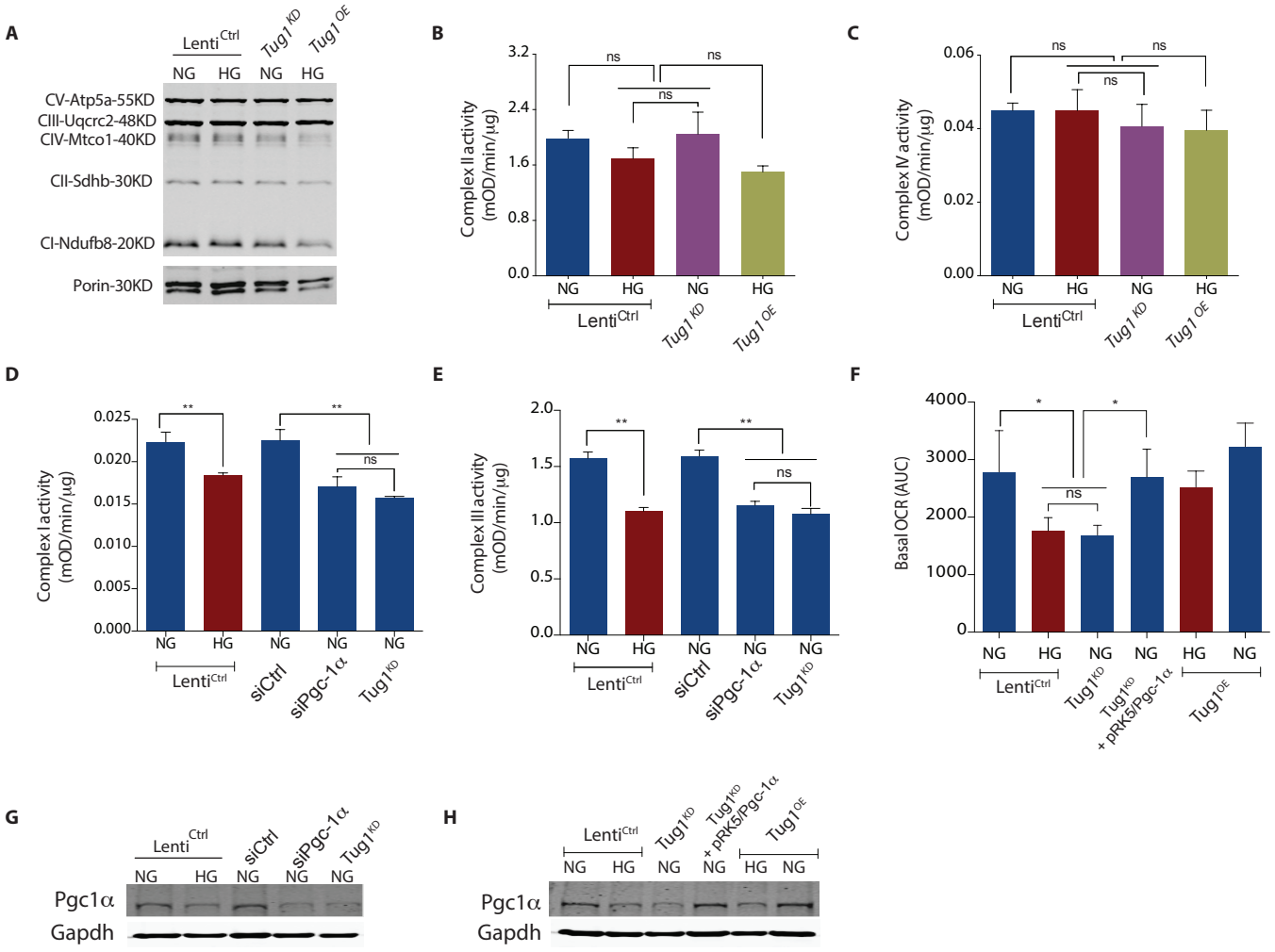
Supplemental Figure 2. Characterization of podocytes-specific *Tug1* transgenic mice . **(A)** qPCR analysis of *Tug1* RNA levels in isolated podocytes and non-podocytes cellular fractions from 5 week old wild type and *Tug1*^{PodTg} mice (*n*=4 mice/group). **(B-D)** Body weight (**B**), fasting blood glucose (**C**), and kidney weight to body weight ratio (**D**) from db/m, db/m;Tug1^{PodTg} mice (n=5-7 mice/group). **(E)**Top, Representative light micrographs from kidney sections stained with antibody directed again Wt1 protein. Bottom, magnification of dashed red box inset from first row highlighting the nuclear loalization and specificity of Wt1 signal. Scale bars denote 50μm. Expression values were normalized to Gapdh. ns: no significance, $^{**}P < 0.01$, $^{***}P < 0.00$, 1-way ANOVA followed by Tukey's post hoc analysis. Data expressed as mean ± SEM.



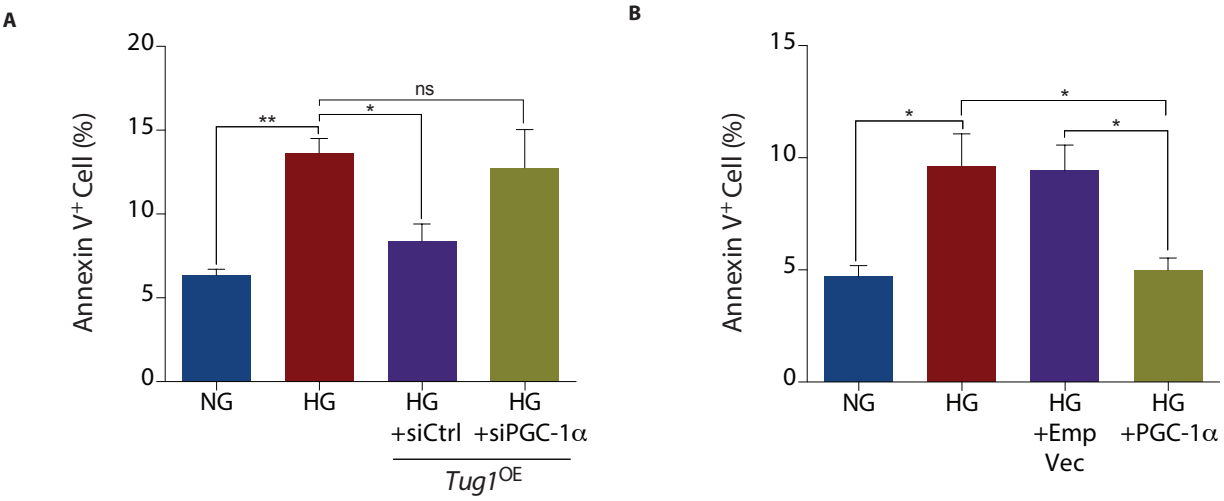
Supplemental Figure 3. CRISPR/Cas9 mediated targeting of *Tug1* expression. (A) Genomic context of *Tug1* (isoform c) promoter and exons. Red arrows denote short guide RNA pairs that were selected to target *Tug1* using CRISPR/Cas9 mediated targeting. **(B)** Construct design highlighting the location of each sgRNA driven by a unique short RNA promoters (mouse U6, human U6, H1 and 7SK) cloned into a lentiviral construct harboring either Cas9D10A (Nickase) to knockdown (KD) *Tug1* expression (top) or a catalytically inactive Cas9 mutant fused with a VP64 activation domain to overexpress (OE) *Tug1* (bottom). **(C-D)** qPCR validation of *Tug1* RNA levels in *Tug1* KD or *Tug1* OE clones expanded under puromycin selection following FACS sorting. Controls were wild-type, non-targeted podocytes transduced with an empty control vector and undergone selection. Values were normalized to Gapdh internal controls. $^{**}P<0.01$,$^{***}P<0.001$. 1-way ANOVA followed by Tukey's post hoc analysis. Data are presented as mean±SEM.



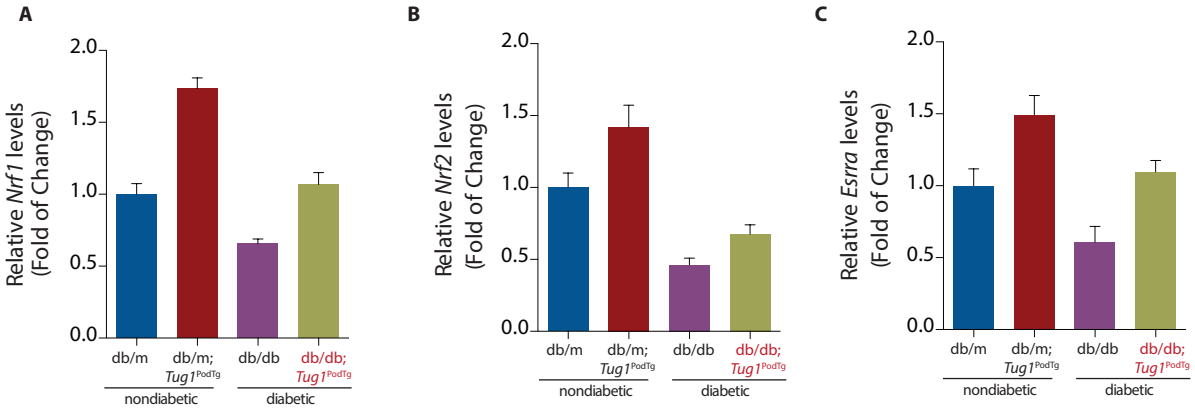
Supplemental Figure 4. Regulation of PGC-1α and its target genes by *Tug1* in multiple cell lines. qPCR analysis of RNA levels of *Tug1*, *Pgc1α* and the indicated target genes in C2C12 **(A)** and AML12 **(B)** cells following depletion of Tug1 RNA using either shRNA (C2C12) or siRNA (AML12) methods. Expression values were normalized to Gapdh. ns: no significance, $^{**}P < 0.01$, $^{***}P < 0.00$, 1-way ANOVA followed by Tukey's post hoc analysis. Data are presented as mean±SEM.



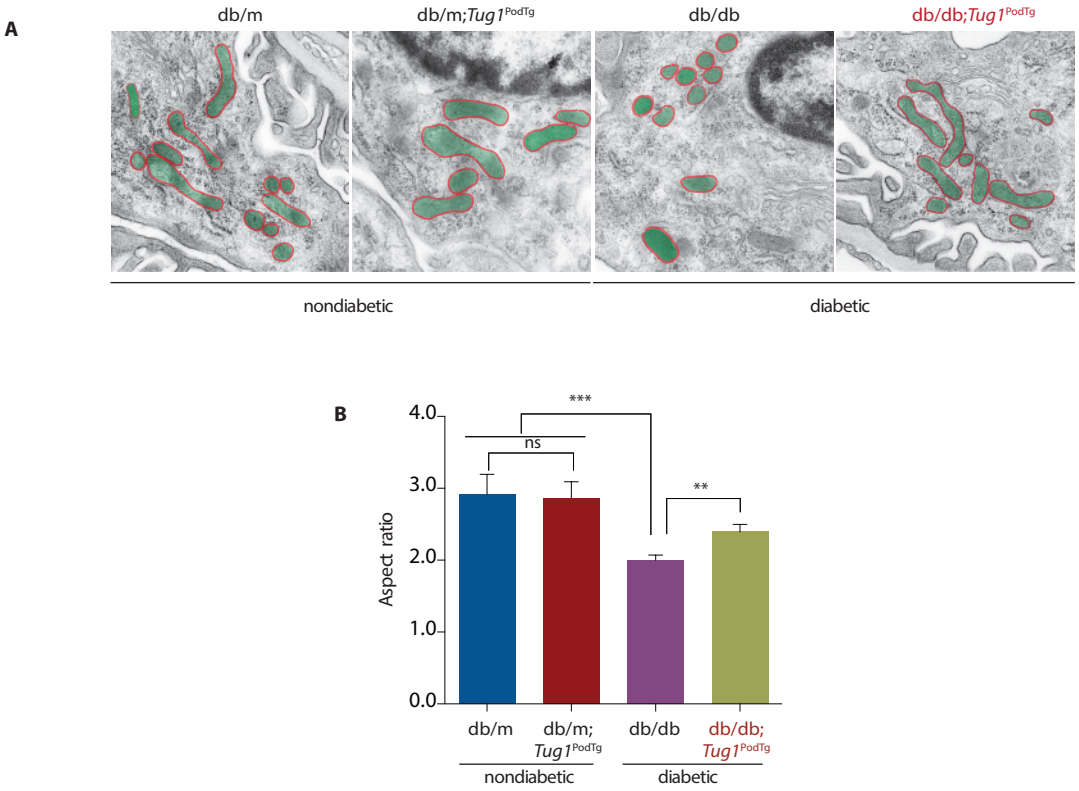
Supplemental Figure 5. Complex I/III activity but not II/IV are regulated by Tug1-PGC-1α. (A) Immunoblot of mitochondria complex subunits in mitochondria isolated from cultured podocytes, using Total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam), Porin is the loading control for mitochondria. **(B-C)** Measurement of Complex II and IV activity in mitochondria isolated from cultured podocytes, measured as rate of change in Optical Density (OD) normalized to total protein content. **(D-E)** Measurement of Complex I and III activity in mitochondria isolated from cultured podocytes transfected with indicated siRNA and treated with different glucose conditions, measured as rate of change in Optical Density (OD) normalized to total protein content. **(F)** Measurement of basal oxygen consumption rate of cultured podocytes under the indicated transfection and glucose conditions, normalized to cell number. **(G-H)** Immunoblot analysis of Pgc1α or Gapdh (loading control) in whole cell lysate from podocytes utilized for complex activity **(D,E)** and Seahorse bioanalyzer assays **(F)**. ns: no significance, $^{**}P < 0.01$, $^{***}P < 0.00$, 1-way ANOVA followed by Tukey's post hoc analysis. Data are presented as mean±SEM.



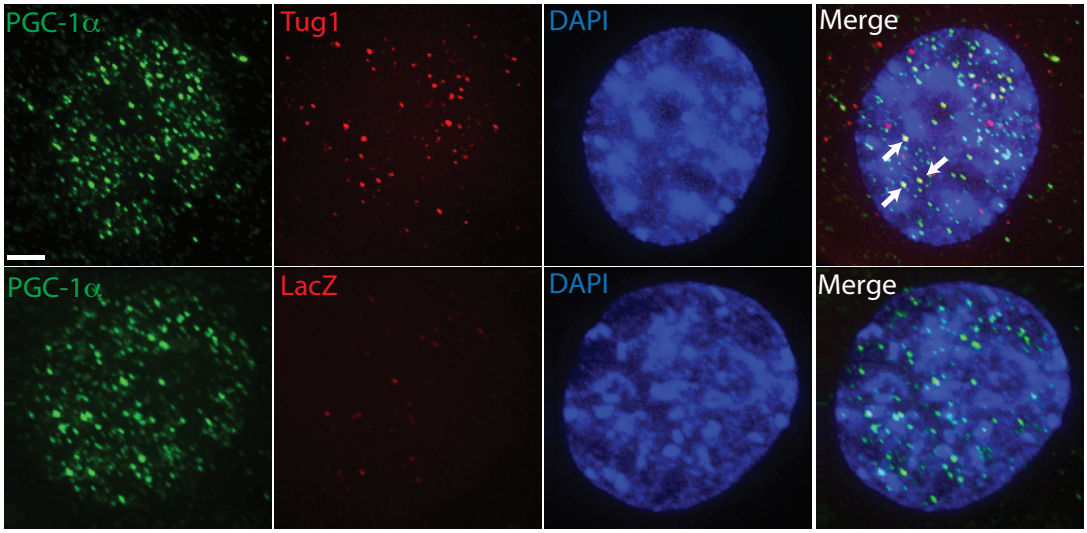
Supplemental Figure 6. Effect of PGC-1α on HG-induced apoptosis in podocytes (A) Podocytes stably overexpressing *Tug1* were transfected with PGC-1α siRNA or a nontargeting control siRNA and cultured under HG conditions. % apoptotic cells, as measured by Annexin-V FITC$^+$ cells, were quantified using flow cytometry. Controls included non mutant podocytes cultured under NG or HG conditions **(B)** Podocytes were transfected with pRK5 empty vector or a pRK5/PGC-1α and cultured under HG conditions. % apoptotic cells, as measured by Annexin-V FITC$^+$ cells, were quantified using flow cytometry. Controls included normal podocytes cultured under NG or HG conditions. ns: no significance *$P < 0.05$, **$P < 0.01$. 1-way ANOVA followed by Tukey's post hoc analysis. Data are presented as mean±SEM.



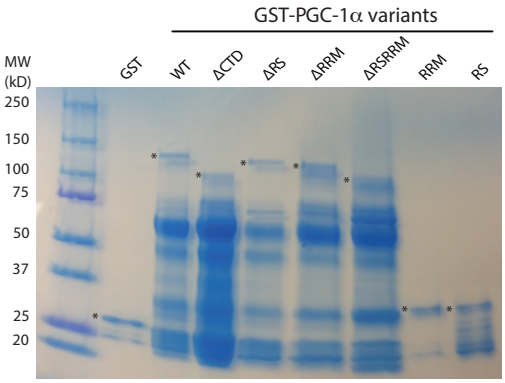
Supplemental Figure 7. Expression of PGC-1α targets is affected in podocytes-specific *Tug1* transgenic mice. qPCR analysis of *Nrf1* (**A**), *Nrf2* (**B**) and *Esrra* (**C**) RNA levels in podocytes from db/m, db/m;Tug1^{PodTg} , db/db and db/db;Tug1^{PodTg} mice (*n*=3 mice/group). Expression values were normalized to Gapdh. Data are presented as mean±SEM.



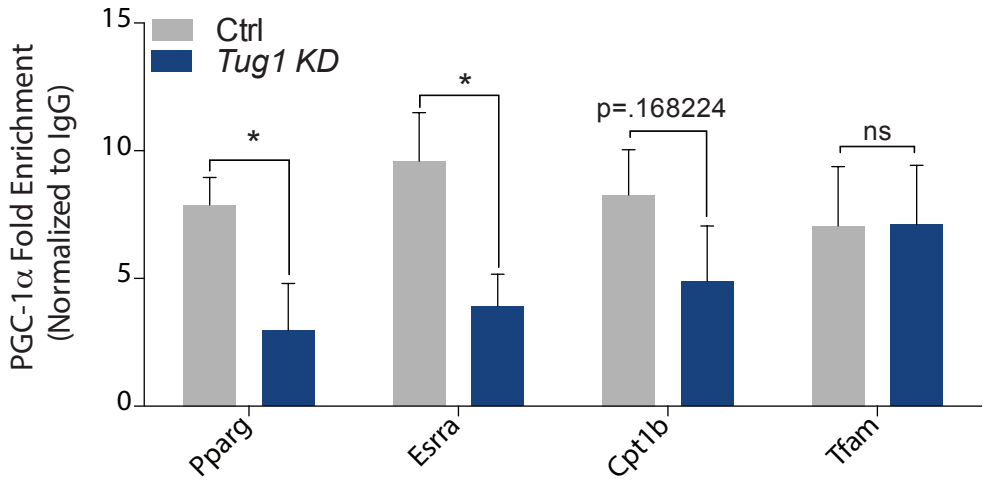
Supplemental Figure 8. Mitochondrial fission is attenuated in podocytes-specific diabetic *Tug1* transgenic mice . (A) Transmission electronmicrographs of podocyte cell bodies from wild type and Tug1^{PodTg} nondiabetic and diabetic mice. Wild type (WT) diabetic mice display mitochondria which are undergoing significant mitochondrial fission, whereas mitochondria from Tug1^{PodTg} are more elongated (*n*=2-3 mice/group). Green outlined miochondria are meant to highlight individual mitochondrion shape. **(B)** The extent of mitochondrial fission was quantified by the aspect ratio measurement of 50 individual mitochondria from TEM micrographs from mice for earch group. ns: no significance, $^{**}P < 0.01$, $^{***}P < 0.00$, 1-way ANOVA followed by Tukey's post hoc analysis. Data expressed as mean ± SEM.



Supplemental Figure 9. Fluorescent in situ hybridization and immunofluorescence demonstrating interaction between Tug1 and PGC-1α in intact podocytes. Single molecule RNA fluorescent in situ hybridization against full length Tug1 (red) coupled with immunofluorescene against PGC-1α protein (green) in cultured mouse podocytes. Cells were stained with probes designed to target LacZ as a negative control for the in situ hybridization. Nuclei were counter-stained with DAPI. White arrows represent merge between Tug1 and PGC-1α signal. Scale bars denote 10 μm.



Supplemental Figure 10. Coomassie brilliant blue staining of SDS-PAGE gel with GST and GST-PGC-1α variants. Protein/RNA complex samples from beads after *in vitro* binding of *Tug1* with indicated GST proteins as in Figure 7G were separated on 4-20% SDS-PAGE and stained with Coomassie brilliant blue, to show comparable GST proteins amount in loading. * denotes the expected band of GST proteins according to its size.



Supplemental Figure 11. ChIP-qPCR against PGC-1α target genes in *Tug1* KD podocytes. ChIP-qPCR analysis of PGC-1α in control wild-type (Ctrl) and *Tug1* KD cells with primers designed to detect the proximal promoter regions of *Pparg*, *Esrra*, *Cpt1b* and *Tfam*. Data are presented as fold enrichment normalized to IgG. ns: no significance, **P* < 0.05, two-tailed student's t-test, Data expressed as mean ± SEM.

Supplemental Experimental Procedures

Functional analysis of TBE in luciferase assays. TBE (Tug1 binding element) at 400kb upstream of mouse *Ppargc1a* gene was identified by CHIRP-Seq. The 380bp AG repeats-enriched TBE was PCR amplified from a BAC clone (clone ID CH29-18E6, CHORI, Oakland, CA) using Herculase II Fusion DNA Polymerase (Agilent, Santa Clara, CA). TBE in sense and antisense were both subcloned into mouse PGC-1α 2kb promoter luciferase construct (Addgene, Cambridge, MA) (1) or the empty pGL3-Basic vector (Promega, Madison, WI). Transient transfection and luciferase reporter assays in cultured mouse podocytes were carried out as previously described (2, 3), using Steady-Glo Luciferase Assay System (Promega, Madison, WI) normalized to β-gal activity.

Subcellular fractionation of Tug1 RNA. Subcellular fractionation of podocytes was performed using CelLytic NuCLEAR Extraction Kit (Sigma). RNAs were then isolated from both nuclear and cytoplasmic fractions. 1 μg of RNA was used for qRT-PCR analysis of Tug1, noncoding RNA Neat2 and U6 snRNA (both are nuclear retained) and β-actin mRNAs (exported to cytoplasm). Values represent the median of 4 technical replicates ± SEM for qRT-PCR.

RNA immunoprecipitation (Continued). For endogenous RIP assay using RNA probes, biotin-labeled *Tug1* RNA and antisense-*Tug1* were *in vitro* transcribed with the Biotin RNA Labeling Mix (Roche, Indianapolis, IN) and SP6 RNA polymerase (Promega) and purified with NucAway Spin Columns (Thermo Fischer Scientific). 5 μg RNA was heated to 90 ºC for 2 min, chilled on ice for 2 min, supplied with 1× RNA structure buffer (10 mM Tris pH 7, 0.1 M KCl, 10 mM MgCl2), and shifted to room

temperature for 20 min to allow proper secondary structure formation. Nuclear lysate was pre-cleared by incubation with 40 μl of avidin agarose beads (Thermo Fischer Scientific) at 4°C for 1 hr. The cleared lysate was incubated with 40 μl of Streptavidin Dynabeads M-280 at 4°C overnight. After five times wash with NETN buffer and one time with 1× RNA structure buffer, RNA/protein complex was eluted with 1× SDS sample buffer and separated on SDS-PAGE. To detect *Tug1*/PGC-1α interaction in cultured cells, HEK 293T cells were transfected with empty vector, or Flag-PGC1α wild type or delta CTD (Addgene, Cambridge, MA) (4). Cleared lysates were prepared after cross-linking and immunoprecipitated with anti-Flag M2 affinity gel (Sigma) as above. Fold enrichment of Tug1 Flag-PGC1α variants transfection normalized to empty vector was calculated. To detect *Tug1*/PGC1α interaction *in vitro*, wild type mouse PGC1α or its deletion mutant cDNAs were amplified by PCR from Flag-PGC1α and subcloned into pGEX2TK (GE Healthcare, Pittsburgh, PA). GST or GST-PGC1α variants were expressed and purified from *E. coli* using Glutathione Sephrose-4B (GE Healthcare) as previously described (5). *Tug1* or *luciferase* was *in vitro* transcribed from the vector pRK5 using MAXIscript SP6 Transcription Kit (Promega). 5 μg folded RNAs were mixed with 2μg of GST proteins immobilized on the beads in 500μl of RIP lysis buffer. RNA/protein complex were purified as above, and fold enrichment of Tug1 in GST-PGC1α variants normalized to GST was calculated.

Seahorse metabolic analyzer assays. Cultured podocytes were grown on 0.1μg/ml collagen-coated plates in RPMI-1640 media supplemented with 10% FBS, 1% Anti-

Anti and maintained in HG (25mM) or normal glucose (5 mM) conditions. Cells were passaged to a 24 well XF24 plate (Seahorse Biosciences) at a cell density of 5×10^4 cells/well. Cartridge plates for metabolic stress injections were hydrated for at least 8 hours at 37°C prior to the assay with calibrant solution (Seahorse Biosciences). One hour prior to running the seahorse assay, the XF24 plate's running medium was removed and replaced with Seahorse Assay Medium (final volume 500 µl per well). Assay conditions and set up were performed according to instructions described by Seahorse Biosciences. OCR was reported in the unit of picomoles per minute and the results were normalized to cell number using CyQUANT Direct Cell Proliferation Assay kit (Molecular Probes). The area under the curve was calculated for the area as previously described (6).

Mitochondrial complexes activity. Mitochondria were first isolated from cultured podocytes using Mitochondria Isolation Kit for Cultured Cells (Thermo Fischer Scientific) following manufacturer's instructions. Activities of respiratory chain complexes in mitochondria were measured in microplates using the specific kits ab109721 (complex I), ab109908 (complex II), ab109905 (complex III) and ab109911 (complex IV) from Abcam (Cambridge, UK) normalized to protein concentration as measured by DC Protein Assay (BioRad).

Mitochondrial aspect ratio. Mitochondrial fission and aspect ration were measured as previously described (7). Briefly, digital images collected by transmission electron microscopy were filtered with a 7×7 hat filter to segment individual mitochondria. To determine mitochondrial fission, the lengths of individual mitochondria were measured by tracing using an image analysis system (NIS

Elements, Nikon). For each group at least 50 podocyte images from three different animals were counted. The morphology of mitochondria was quantified by determining the area and the best-fitting ellipse yields the longitudinal length (major), equatorial length (minor), and angle between major and minor for each organelle. Subsequently, the average of these parameters was calculated and used for the calculation of the aspect ratio to evaluate the elongation of mitochondria (Aspect ratio=major axis/minor axis).

Cell apoptosis, mitochondrial ROS and ATP determination. For Annexin V FITC labeling, podocytes were grown in 6 well plates until they were 70% confluent. The cells were then serum starved overnight followed by treatment with NG or HG for 48hrs. The cells were incubated at 37°C with fresh media containing 5μM MitoSox Red mitochondrial superoxide indicator (Invitrogen). The cells were washed once in PBS and incubated with Annexin V FITC (Life Technologies) according to the manufacturer's instruction. The cells were incubated with DAPI (1 μg/mL) for 5 min on ice and then analyzed by flow cytometry (FACS Aria, Becton Dickinson, San Jose, CA). This assay discriminates between intact cells, early apoptotic cells, and late apoptotic or necrotic cells. Cellular ATP levels were measured using the CellTitre-Glo Luminescent Cell Viability Assay Kit (Promega), as previously described (8).

Mitochondrial DNA estimation. Mitochondrial DNA copy number was estimated as previously described (9). Briefly, the total DNA from cultured or isolated mouse podocytes were isolated using DNeasy Tissue Kit (Qiagen) according to manufacturer's instructions. Quantitative PCR was performed by using equal amount of DNA from each samples, Power SYBR Green Master Mix (LifeTech,

Garland Island, NY) and 5μM of each primers, i.e. mouse mitochondrial ND1, mouse mitochondrial Cyt B, and mouse nuclear H19 DNA. To compare mitochondrial copy number, ratio of ND1:H19 and/or Cyt B:H19 was calculated. Results are presented as percent change in the mitochondrial DNA in experimental versus wild-type/control samples.

Sequential immunofluorescence and fluorescent in situ hybridization Cultured podocytes were differentiated on collagen-coated coverslips for 14 days after which cells were fixed washed twice with 1x PBS and fixed with freshly prepared 4% paraformaldehyde. Cells were permeabilized with 0.1% Triton X-100 in 1x PBS for 5 minutes at room temperature. After washing once with 1x PBS, PGC-1α antibody (NBP1-04676, Novus, 1/100) was diluted in 1x PBS and applied to cells for 1 hour at room temperature. FITC conjugated rabbit secondary antibody diluted in 1x PBS was applied for 1 hour at room temperature. Cells were washed and post-fixed with 4% paraformaldehyde for 10 minutes at room temperature. Cells were washed once with 1x Wash Buffer A (5x Stellaris RNA FISH Wash Buffer A (Biosearch Technologies, SMF-WA1-60), 10x deionized formamide and RNase-free dH_2O) for 5 minutes. 125nM of Tug1 or LacZ control probe diluted in hybridization buffer (10% deionized formamide in Stellaris RNA FISH Hybridization Buffer (Biosearch Technologies, SMF-HB1-10) was applied to the cells and incubated overnight at 37° C in the dark. Cells were washed once in Wash Buffer A for 30 minutes at 37° C. Cells were counterstained with DAPI diluted in Wash Buffer A for 30 minutes at 37° C. Cells were then washed with Wash Buffer B (Biosearch Technologies, SMF-WB1-20) for 5 minutes at room temperature. A small of drop of Prolong Gold Antifade

mounting medium was applied to each coverslips and were mounted onto slides, sealed with nail polish and immediately imaged on the Deltavision Deconvolution microscope (Applied Precision, LLC, Issaquah, WA).

Chromogenic in situ hybridization Formalin-fixed paraffin embedded tissue sections were dewaxed in xylenes, and rehydrated through an ethanol dilution series. Tissue sections were digested with 10µg/mL proteinase K for 10 minutes at RT and then loaded onto the Ventana Discovery Ultra device for *in situ* hybridization analysis. Tissue slides were hybridized with double-DIG labeled mercury LNA probes (Exiqon) for 2 hrs at 50 C (Ventana Discovery Ultra). Detection was performed using a polyclonal anti-DIG antibody and Alkaline Phosphatase conjugated second antibody (Ventana) using NBT-BCIP as the substrate. Images were acquired on a Nikon Eclipse 50i microscope.

Biochemical, histological and morphometric studies. Biochemical assays, histological staining, morphometric analyses, serum creatinine measurements and blood pressure measurements were performed as previously described (7). For histological and immunofluorescence analysis, investigators analyzing corresponding data were blinded to the treatment for each individual sample. PAS, TEM and SEM data was examined by an independent pathologist, blinded to the experimental conditions.

Glomeruli and podocyte isolation. Glomeruli were isolated using magnetic Dynabeads as previously described (7). Podocytes were isolated as previously described with slight modifications (10). Following endothelial cell depletion with selection by biotin-labeled CD31; podocytes underwent positive selection with biotin-labeled

Kirrel3 and podocalyxin antibodies (R&D Systems, Minneapolis, MN). Podocytes were then isolated from the total cell population using magnetic, streptavidin labeled Dynabeads (Thermo Fischer Scientific).

Chromatin Immunoprecipitation and qPCR (ChIP-qPCR). ChIP assays were performed according to publicly available protocols obtained from University of Washington Encode program. Briefly, 100 μg of BioRuptor (Diagenode, Belgium) sonicated chromatin extract was incubated with 5 μg of an antibody against PGC-1α (NBP1-04676, Novus) or normal rabbit IgG antibodies (sc-2027, Santa Cruz). After purification of DNA, gene promoter specific sequences were amplified by quantitative real-time PCR. Data were calculated based on fold enrichment of PGC-1α pulldown versus IgG pulldown.

RNA-Seq. RNAs from mouse podocytes were isolated as indicated below. Biological replicate libraries were prepared for downstream analysis. The Illumina compatible libraries were prepared using Illumina's TruSeq RNA Sample Prep kit v2, as per the manufacturer's protocol. In brief, Poly-A RNA was enriched using Oligo-dT beads. Enriched Poly-A RNA was fragmented to a median size of 150bp using chemical fragmentation and converted into double stranded cDNA. Ends of the double stranded cDNA were polished, 5'-phosphorylated, and 3'-A tailed for ligation of the Y-shaped indexed adapters. Adapter ligated DNA fragments were PCR amplified, quantified and validated by qPCR, and sequenced on Illumina's HiSeq 2000 (Illumina Inc., San Diego, CA, USA) in a paired-end read format for 76 cycles. The libraries were sequenced on a version 3 TruSeq paired end flowcell according to manufacturer's instructions at a cluster density between 750 – 1000 K

clusters/mm^2. The samples containing 4 libraries per lane were sequenced for paired end 75 nt with a 7 nt read for indexes using version 3 sequencing reagents. The resulting BCL files containing the sequence data were converted into ".fastq.gz" files & individual sample libraries were demultiplexed using CASAVA 1.8.2 with no mismatches.

RNA isolation and qPCR. Isolation of RNA from cultured cells and tissue was performed using Trizol Reagent (Thermo Fischer Scientific). RNA lysates were further purified on spin column using PureLink RNA Mini kit (Thermo Fischer Scientific) and treated with RNase-free DNase I (NEB Biolabs). Total RNAs were quantified using a Nanodrop 2000 and samples with a 260/280 ratio between 1.9-2.1 were used for downstream applications. RNAs were reverse transcribed into cDNA using the iScript Reverse Transcription Supermix (BioRad, Hercules, CA). cDNAs were subjected to gene specific amplification with primers listed in Supplemental Table 1. qRT-PCR's were performed on StepOne Plus Real Time System (Thermo Fischer Scientific) using 2x Power SYBR Green Master Mix (Thermo Fischer Scientific). Gapdh was used as internal controls for mRNA and lncRNAs.

Microarray. Total RNA from was quantified using the NanoDrop ND-1000 and RNA integrity was assessed by standard denaturing agarose gel electrophoresis. For microarray analysis, Agilent Array platform was employed. The sample preparation and microarray hybridization were performed based on the manufacturer's standard protocols. Briefly, total RNA from each sample was amplified and transcribed into fluorescent cRNA with using the manufacturer's Agilent's Quick

Amp Labeling protocol (version 5.7, Agilent Technologies). The labeled cRNAs were hybridized onto the Whole Mouse Genome Oligo Microarray (4 x 44K, Agilent Technologies). After having washed the slides, the arrays were scanned by the Agilent Scanner G2505C. Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. Quantile normalization and subsequent data processing were performed using the GeneSpring GX v12.1 software (Agilent Technologies). After quantile normalization of the raw data, genes that at least 6 out of 6 samples have flags in Detected ("All Targets Value") were chosen for further data analysis. Differentially expressed genes with statistical significance were identified through Volcano Plot filtering. Pathway analysis and GO analysis were applied to determine the roles of these differentially expressed genes played in these biological pathways or GO terms. Finally, hierarchical clustering was performed to show the distinguishable gene expression profiling among samples.

Scanning electron microscopy. Samples were treated with a fixative containing 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3, overnight at 4°C. The samples were washed with 0.1 M cacodylate buffer, pH 7.3 for 3 x 10 min. The samples were then post fixed with 1% cacodylate buffered osmium tetroxide for 1 hour, washed with 0.1M cacodylate buffer for 3 x 10 min., then in distilled water, 2 x 5 min. The samples were then dehydrated with a graded series of increasing concentrations of ethanol for 5 min each. The samples were then transferred to graded series of increasing concentrations of hexamethyldisilazane (HMDS) for 5 min each and air dried overnight. Samples were mounted on to double-stick carbon tabs (Ted Pella. Inc., Redding, CA), which have been previously

mounted on to aluminum specimen mounts (Electron Microscopy Sciences, Ft. Washington, PA). The samples were then coated under vacuum using a Balzer MED 010 evaporator (Technotrade, NH) with platinum alloy for a thickness of 25 nm., then immediately flash carbon coated under vacuum. The samples were transferred to a desiccator for examination at a later date. Samples were examined in a JSM-5910 scanning electron microscope (JEOL, USA, Inc., Peabody, MA) at an accelerating voltage of 5 kV.

Transmission electron microscopy. Fixed samples were washed in 0.1 M cacodylate buffer, postfixed with 1% buffered osmium tetroxide for 1 hour, and stained en bloc with aqueous 1% Millipore-filtered uranyl acetate. The samples were washed several times in water, then dehydrated in increasing concentrations of ethanol, infiltrated, and embedded in LX-112 medium. The samples were polymerized in a 60°C. oven for about 3 days. Ultrathin sections were cut in a Leica Ultracut microtome (Leica, Deerfield, IL), stained with uranyl acetate and lead citrate in a Leica EM Stainer, and examined in a JEM 1010 transmission electron microscope (JEOL, USA, Inc., Peabody, MA) at an accelerating voltage of 80 kV. Digital images were obtained using AMT Imaging System (Advanced Microscopy Techniques Corp, Danvers, MA).

Western blot analysis. Cell pellets or mitochondria isolated from podocytes according to manufacturer's instructions (ThermoFisher Scientific, Waltham, MA) were lysed in RIPA Buffer (50 mM Tris-HCl, pH 8.0, with 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% SDS). Protein concentration was determined by DC Protein Assay (Bio-Rad, Hercules, CA). Twenty

μg of total protein lysate was diluted in 5x Laemmli buffer, loaded on 4-20% gradient SDS-PAGE (BioRad, Hercules, CA) and transferred to nitrocellulose membranes. Membranes were probed with the indicated antibody followed by visualization by Odyssey Infrared Imaging System (Li-Core Biosciences). The following antibodies were used: anti-Flag (F1804, Sigma, 1:1000), anti-PGC-1α (NBP1-04676, Novus, 1:500, and ab54481, Abcam, 1:250), Total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam, 1:250)). Anti-Gapdh (MAB374, Millipore, 1:2000) or anti-Porin (MSA03, MitoSciences, 1:1000) was used as loading control.

Immunofluorescence. 4 μm thick formalin fixed, paraffin embedded kidney sections were deparaffinized and rehydrated through an ethanol series with a final wash in distilled water. Heat mediated antigen retrieval was performed with Sodium Citrate Buffer (10mM Sodium Citrate, pH 6.4) was performed. After cooling for 20 minutes at room, sections were washed twice with 1x TBS and non-specific binding was blocked with 10% normal donkey serum (Millipore) for 1 hour at room. Podocytes with primary antibodies directed against nephrin (Fitzgerald Laboratories, 1:200) diluted in 10% NDS, overnight at 4°C, and then washed three times with 1x TBS. Fluorophore conjugated secondary antibodies to guinea pig (1:200) were applied to their and incubated at room temperature for 1hr in the dark. Sections were washed three times with TBS and nuclei were counterstained with DAPI (Thermo Fischer Scientific) and mounted with ProLong Gold Antifade mounting reagent (LifeTech, Garland Island, NY). Sections were processed and imaged on a Nikon A1-Rs Inverted Laser Scanning Confocal Microscope. Images were quantified using, NIH ImageJ version 2.00.

Bioinformatics analysis. RNA-Seq- The raw data quality was assessed using FastQC software. Adaptor presence was tested using Trimmomatic. Reads from each sample were aligned to the NCBI mouse reference genome build 37.2 using Tophat2 v2.0.4. Transcript quantification, normalization and assembly were carried out using Cufflinks (11). Cuffdiff2 was used to identify features differentially expressed between conditions. A 0.05 FDR was used in selecting significant genes. To further define differentially regulated genes, a cutoff of $-\log_{10}$(p-value)>1 and a $\log_2$ fold change (FC) of <0.5 or >0.5 was employed.

Microarray- After quantile normalization of the raw data, genes that at least 6 out of 6 samples have flags in detected ("All Targets Value") were chosen for further data analysis. Differentially expressed genes with statistical significance were identified through Volcano Plot filtering. Pathway analysis and GO analysis were applied to determine the roles of these differentially expressed genes played in these biological pathways or GO terms. Finally, Hierarchical Clustering was performed to show the distinguishable gene expression profiling among samples.

ChIRP-Seq- After the sequencing platform generated the sequencing images, the stages of image analysis and base calling were performed using Off-Line Basecaller software (OLB V1.8). After passing Solexa CHASTITY quality filter, the clean reads were aligned to mouse genome (UCSC mm10) using BOWTIE software (V2.1.0). Aligned reads were used for peak calling of the ChIRP regions using MACS V1.4.0. Statistically significant ChIRP-enriched regions (peaks) were identified by comparison to input, using a p-value threshold of 10^{-5}. Peaks were annotated by the nearest gene using the newest UCSC RefSeq database. Peaks located within -2Kb to

+2Kb around the corresponding gene further classified as located within the promoter region of the corresponding gene. Pathway analysis and GO analysis were applied to peaks to determine the roles played in certain biological pathways or GO terms. The signal profile (at 10 bp resolution) with UCSC WIG file format was generated from ChIRP-seq data, which can be visualized on UCSC genome browser or IGB browser (Integrated Genome Browser).

SUPPLEMENTARY TABLE Oligonucleotide Sequences

qPCR primers

Name	Forward primer(5'-3')	Reverse primer (5'-3')
Gapdh	GCCTGGAGAAACCTGCCAA	CGAAGGTGGAAGAGTGGGAG
Tug1	CATAGTATCATCTTCGGGTTAC	CACAAAATGCATGTAGGTTC
Ppargc1a	TCCTCTTCAAGATCCTGTTAC	CACATACAAGGGAGAATTGC
Tfam	GACCTCGTTCAGCATATAAC	ACAAGCTTCAATTTTCCCTG
Esrra	CTTAATCCGATCTCCTCTCC	TCAGATTTGGAAGCAGTTTG
Nrf1	AAACAAAGGGTTTCATGGAC	GGTACGAGATGAGCTATACTG
Gabpa (Nrf2)	CAAGTATTGACAGTACCAGC	CTCTCTCTTCAATTTCTGCAC
Neat2	TGCAGTGTGCCAATGTTTCG	GGCCAGCTGCAAACATTCAA
Actb	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA
Rnu6	CGCTTCGGCAGCACATATAC	TTCACGAATTTGCGTGTCAT
Pdk4	ACAATCAAGATTTCTGACCG	TCTCCTTGAAAATACTTGGC
Cpt1b	ACTAACTATGTGAGTGACTGG	TGGCATAATAGTTGCTGTTC
luciferase (luc)	TCAAAGAGGCGAACTGTGTG	GGTGTTGGAGCAAGATGGAT

ChIP primers

Name	Forward primer(5'-3')	Reverse primer (5'-3')
TBE	CCTCTTTCCCTTTCAGTCTCC	CCACCTTGAACACTATTCCTGA
intergenic	CACTGTGTGGATGGTTTTGC	TCAGTGCCCCAGAAGAGACT
distal	TTTGAGGCCCAAGAAACAAG	GGCTGATGAACACAGACACG
medial	GAGAGAAAGAAAATCGGGGG	ATGCCACCAACTCTAAACCG
proximal	GCATGAGTGTGTGCTGTGTG	TCCAGGAATCATTGCATCTG
Pdk4	TGATTGGCTACTGTAAAAGTCCCGC	ATCCCAGGTCGCTAGGACTTCAGG
Pparg	AATCTCTGCTCTGGTAATTCCAAC	AAATGGTGTGTCATAATGCTGTCT
Esrra	GCCTTTCATTTACATACACGTTCA	GGAGGAGCGAAGACTAGGAACT
Cpt1b	CAGCTATTTTTAGGACTGATGCAA	AGAAAACAGATAGGGGTGGAAAC
Tfam	AGCAGTACCACGGATCTCTAACTT	GTCTAAGGTGGGTGTTGCTGAG

Mitochondrial DNA copy number primers

Name	Forward primer(5'-3')	Reverse primer (5'-3')
mouse ND1	CTCTTATCCACGCTTCCGTTACG	GATGGTGGTACTCCCGCTGTA
mouse CytB	GTGAACGATTGCTAGGGCC	CGATTCTTCGCTTTCCACTTCAT
mouse H19	GTACCCACCTGTCGTCC	GTCCACGAGACCAATGACTG

gRNAs targeting Tug1

	sequence (PAM sequence highlighted)	targeting strand/region
sg-1a	GACGTCGCTCGCCAATCAGA**AGG**	antisense/promoter
sg-1b	CGGTCCGCCCCCATCACGTG**AGG**	sense/promoter
sg-2a	ATGAGGAATTCAAAATGCCT**TGG**	antisense/exon 3
sg-2b	GTATCATCTTCGGGTTACTC**AGG**	sense/exon 3

gRNAs targeting TBE

	sequence (PAM sequence highlighted)	targeting strand
hU6-gRNA	ATAGTGTTCAAGGTGGAATAA**AGG**	sense
mU6-gRNA	TCTCTCTCTCATCTACTTTT**TGG**	sense
7SK-gRNA	CTACTTAGTATCTACTGTTC**AGG**	antisense
H1-gRNA	GGATTTTTTCCAAAGGACGA**GGG**	sense

Tug1 transgenic mice genotyping primers

Name	Forward primer(5'-3')	Reverse primer (5'-3')
Tg-geno	CACCCGACGGTCTTTAGGGT	CCTTCTCCAGAGGAAAGCCT

ChIRP probes

Name	sequence
Tug1_01	TCTCTGGCCGAGAAGAAAGA
Tug1_02	TCTTTTGAGGTACATCCAGA
Tug1_03	TATGAACCTCCAATCCTGGA
Tug1_04	TTCTTGAAAATCTGGGAGCC
Tug1_05	TCTTTTCTCACGGCAAGATC
Tug1_06	TGTTGTCAGTTTGCACTGAG
Tug1_07	GGAGTAGCAGAAGGAAGGTC
Tug1_08	ACTGGAATGGTGTCTTCTTC
Tug1_09	TGAATCTGGGCAGAGTAGGA
Tug1_10	TTTATGGAATAAACCTGGCC
Tug1_11	AATGCTGTTCTCAGAGATGC
Tug1_12	AAGAACAAGAGTCCAGTGGC
Tug1_13	AAATGCCTTGGTTTTGATCC
Tug1_14	CAAGAGGCCTTTTCAGGAAT
Tug1_15	CTTCACTACTTCATCCAGAG
Tug1_16	TCCAGGTCAAACATGTTGTC
Tug1_17	CTCTCTGGTTATATCCAACT
Tug1_18	GACTTCCAAGACACGTACTT
Tug1_19	CTGCTCCATAGTTAATGAGG
Tug1_20	CTGGTTGCATCAACATGACT
Tug1_21	GTGGTGAGTAGTTACTTTTG
Tug1_22	GGAAAATTTCCAGCCACATT
Tug1_23	GTCAAACTTTGCCTATCACG
Tug1_24	CTCTACTTTTCCAGCAAACA
Tug1_25	TAAGGAGTCAGTCACTCTGT
Tug1_26	AGTCTTGTTCACTATGATGA
Tug1_27	GCACTCAGAGTAGCTGATAG
Tug1_28	ATTTGCTGATTTGTGGCGAG
Tug1_29	GTAGTTGCATATACAAGCAG
Tug1_30	GAGTATGGCAGGAGGGAATA
Tug1_31	CTAGATTAAGGCTTTTGCCT
Tug1_32	AGCAAGTCAAGACCTCAGAC
Tug1_33	GTTTGTCAAAGTTGGCTCTA
Tug1_34	AGCTTAGAAGGCTATATCCT
Tug1_35	GGGTTTAGTCCAGACATTAT
Tug1_36	CAAACTACTTCAGTAGGGCC
Tug1_37	GGGCAAAGGAGAGGCTGAAA
Tug1_38	TGCTGGTCAAGGAATATGCA
Tug1_39	TGCTGTCAACCTTCTATACT
Tug1_40	TGCTGTTTCTTGTCAAGGTG
Tug1_41	AAAACTAGCCTCCTACTTGG
Tug1_42	TAGGATTCACCATAAGGTCC
Tug1_43	AATCACCAGGAGTGATGTCT
Tug1_44	TCCAGGATACGACCAGATAT
LacZ_01	CCAGTGAATCCGTAATCATG
LacZ_02	GTAGCCAGCTTTCATCAACA
LacZ_03	ATCTTCCAGATAACTGCCGT
LacZ_04	ATAATTTCACCGCCGAAAGG
LacZ_05	TTCATCAGCAGGATATCCTG
LacZ_06	TGATCACACTCGGGTGATTA
LacZ_07	AAACGGGGATACTGACGAAA
LacZ_08	GTTATCGCTATGACGGAACA
LacZ_09	TGTGAAAGAAAGCCTGACTG
LacZ_10	GTAATCGCCATTTGACCACT

Stellaris FISH Probes **Sequence**

Name	BioSearch Technologies ID:
Tug1	VSMF-3137

Name	sequence
LacZ_01	TGCAAGGCGATTAAGTTGGG
LacZ_02	AATGGGATAGGTCACGTTGG
LacZ_03	GAACAAACGGCGGATTGACC
LacZ_04	ATGTGAGCGAGTAACAACCC
LacZ_05	TAGCCAGCTTTCATCAACAT
LacZ_06	TTGCACCACAGATGAAACGC
LacZ_07	TTCAGACGGCAAACGACTGT
LacZ_08	CGCGTAAAAATGCGCTCAGG
LacZ_09	TCCTGATCTTCCAGATAACT
LacZ_10	GAGACGTCACGGAAAATGCC
LacZ_11	TGTGTAGTCGGTTTATGCAG
LacZ_12	GGCAACATGGAAATCGCTGA
LacZ_13	CGCGGCTGAAATCATCATTA
LacZ_14	CACCCTGCCATAAAGAAACT
LacZ_15	CTCATCGATAATTTCACCGC
LacZ_16	ACGTTCAGACGTAGTGTGAC
LacZ_17	GCACGATAGAGATTCGGGAT
LacZ_18	TAACGCCTCGAATCAGCAAC
LacZ_19	TGACCATGCAGAGGATGATG
LacZ_20	TTCATCAGCAGGATATCCTG
LacZ_21	CACGGCGTTAAAGTTGTTCT
LacZ_22	AGCGGATGGTTCGGATAATG
LacZ_23	GGGTTTCAATATTGGCTTCA
LacZ_24	GATCATCGGTCAGACGATTC
LacZ_25	GATCACACTCGGGTGATTAC
LacZ_26	GGATCGACAGATTTGATCCA
LacZ_27	CGCGTACATCGGGCAAATAA
LacZ_28	AAGCCATTTTTTGATGGACC
LacZ_29	TATTCGCAAAGGATCAGCGG
LacZ_30	GTTGCCGTTTTCATCATATT
LacZ_31	CATACAGAACTGGCGATCGT
LacZ_32	AAACTGCTGCTGGTGTTTTG
LacZ_33	TTTGCCCGGATAAACGGAAC
LacZ_34	CGCTATGACGGAACAGGTAT
LacZ_35	GTAGTTCAGGCAGTTCAATC
LacZ_36	AATTGCCAACGCTTATTACC
LacZ_37	AAAGAAAGCCTGACTGGCGG
LacZ_38	TTGTTTTTTATCGCCAATCC
LacZ_39	ACTTACGCCAATGTCGTTAT
LacZ_40	CGCATCAGCAAGTGTATCTG
LacZ_41	CATCAATCCGGTAGGTTTTC
LacZ_42	CAACGGTAATCGCCATTTGA
LacZ_43	GTCAAAACAGGCGGCAGTAA
LacZ_44	GGAAGACGTACGGGGTATAC
LacZ_45	GTGGGCCATAATTCAATTCG
LacZ_46	TAGCGGCTGATGTTGAACTG
LacZ_47	GGAAACCGTCGATATTCAGC
LacZ_48	ACACCAGACCAACTGGTAAT

Exiqon LNA ISH Probe

Name	
mTug1_1_2	5'-digATGGTGGTAAAGGAAGATGGTdig-3'

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